

Advancing Beyond the Atom-Centered Model in Additive and Nonadditive Molecular Mechanics

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ABSTRACT: A computational approach to the inclusion of off-center charges in both additive and nonadditive molecular mechanics calculations is presented. The additional sites in the molecular skeleton are placed in the approximate locations of the chemically intuitive electron lone pair, and are treated as formal particles throughout the calculation. The increase in the number of charge sites results in overall improvement in the energy associated with the angular dependence of hydrogen bonds and improved statistical accuracy of the electrostatic potential derived charges. The addition of lone pairs also results in improved accuracy in relative solvation free energy calculation for the pyridine to benzene and methanol to methane mutations. Because the number of atoms that require lone pairs is small, the extra accuracy can be achieved with little computational overhead. © 1997 John Wiley & Sons, Inc. *J Comput Chem* **18**: 1632–1646, 1997

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Introduction

The electrostatic energy is often the major contribution to molecular interactions in chemical and biochemical systems.¹ It is crucial to the understanding of reactivity, and molecular processes in general, that electrostatic forces be described as accurately as possible.² In the case of molecular mechanics/dynamics studies of systems of organic and biochemical interest, the total energy is usually described by a set of simple equations, the molecular mechanical force field, empirically parameterized from experimental or quantum mechanical data. The electrostatic portion of the force field is usually of the simple form:

$$E_{elec} = \frac{1}{2} \sum_{i < j}^n \sum_j^n \frac{q_i q_j}{\epsilon R_{ij}} \quad (1)$$

where q_i and q_j are the formal charges associated with atomic centers i and j , R_{ij} is the distance between them, and ϵ is the dielectric constant. An analogous equation is implemented in MM2³ and MM3⁴ for the interaction between point dipoles.

How can one derive the charges $\{q_i\}$ or the analogous point dipoles? There are two main methods: first, one can derive them empirically, based on experiment and/or quantum mechanical calculations (e.g., gas phase hydrogen bonds^{3–6}). Alternatively, one can derive them using an analysis of the quantum mechanical charge distribution, either through distributed multipole analysis (DMA), or by statistically fitting to reproduce the quantum mechanically calculated electrostatic potential outside of the van der Waals envelope of the molecule. Although there are advantages and disadvantages to each approach, as discussed elsewhere,⁷ it is clear that use of electronic structure calculations has the potential to give the more accurate charge distribution. The DMA approach is promising; however, it has not yet reached a level of robustness necessary for use in the development of $\{q_i\}$ for general force field calculations, whereas electrostatic potential derived charges have. It is clear that such electrostatic potential charges can be particularly powerful because they fit very well not only the first nonvanishing multipole of the molecule, but higher order moments as well. The pioneering work in this area^{9,10} was followed up by the derivation of $\{q_i\}$ for use in a protein and nucleic acid force field.^{11,12} By means

of these potential derived charges, force field calculations have been successful in reproducing interaction and conformational energies, liquid properties, and relative free energies of hydration and binding.^{7,12–15}

The organic/biochemical force field derived using 6-31G*¹⁶ electrostatic potential charges⁷ is improved over that presented in Ref. 12, probably because its $\{q_i\}$ is more balanced with respect to models of water (TIP3P¹⁷ and SPC/E¹⁸) used with it. It is our opinion that the most important remaining inaccuracies vis-à-vis the ability of the force field to simulate structures and energies of molecules of organic and biochemical interest are: (1) the use of an effective two-body model to represent many-body effects; and (2) the use of charges centered only on atoms. This latter approximation was chosen for consistency with the above water models. Given the crucial nature of the electrostatic contribution to the total energy, these two inaccuracies appear to be a logical place to attempt to improve the quality of the force field. One of the drawbacks of forcing the charges to be located at the atomic centers is, in general, lower accuracy [19], and, in particular, the lower accuracy of such models in representing directionality of hydrogen bonds [20].

In our earlier force field, drawing on the results of our *ab initio* calculations on $\text{H}_2\text{S} \cdots \text{HF}$,²¹ we did include lone pairs on sulfur. These were not included in our later force field only because sulfur is so rarely a hydrogen bond acceptor in proteins.²² Although force fields often accurately reproduce the lowest energy conformation of hydrogen bonded systems, suboptimal structures can be poorly represented. Average properties such as solvation, liquid structure, and energetics are often handled well, but detailed interactions may suffer. These interactions will be crucial in any problem dependent upon specific atomic interactions, such as protein–ligand design. Take for example the conformational change represented in Figure 1. As can be seen, the force field significantly underestimates the energy cost of this conformational change. To improve the molecular mechanics description of this type of system, it is likely to be necessary to impart some nonspherical character to the hydrogen bond acceptor.

Several alternative methods designed to accomplish this present themselves, some of which substantially increase the computational burden of the underlying model. Such approaches include adding, in a general fashion, higher order electric effects on all atoms.^{19,24} Although this methodol-

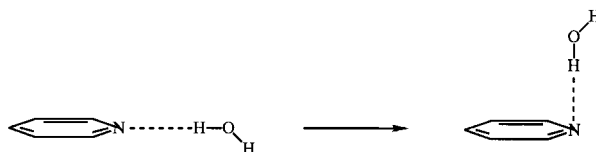


FIGURE 1. Conformational change involving pyridine ... HOH hydrogen bond.

Model	E(tot, 180°)	E(int, 180°)	E(tot, 90°)	E(int, 90°)	ΔE
6-31G*//6-31G*	-322.7155701	-5.65	-322.7102914	-2.34	-3.31
MP2/6-31G*//G-31G*	-323.6884628	-7.61	-323.6821843	-3.67	-3.94
AMBER 4/TIP3P	-9.0951	-5.25	-8.6571	-4.81	-0.44

ogy may be useful in some cases, what is proposed here is a much simpler approach which retains almost all of the power and efficiency of the original force field model, while improving its applicability. In particular, we will delineate the effect of including formal lone pairs in the atomic description of the chemical systems on force field performance. In addition to the simplicity of the proposed model, the added flexibility should allow the use of more realistic charge distributions; that is, ones in which calculated electric moments more closely reproduce experimental measurements, with both additive and nonadditive force fields.

As noted previously, in two-body additive force field development, one seeks a charge distribution that implicitly includes polarization effects. This is the logic behind the choice of the 6-31G* basis set for electrostatic potential generation,⁷ in spite of its consistent overemphasis of dipole moments. However, as force fields go beyond the two-body approach, and include polarization, it will be essential to have a more precise charge distribution that reproduces more than just the first nonvanishing multipole moment. For example, an atom-centered model is inherently incapable of reproducing both the dipole and quadrupole moment of the water molecule.²⁵ If the restriction is made that calculated electric moments must be accurate, lone pairs will be one of the few ways in which interaction energies and hydrogen bond directionality can be fine tuned in a force field, particularly a nonadditive one. The results presented will more clearly illustrate this point.

It should be pointed out that lone pairs have been included in many treatments of electrostatics prior to this investigation.²⁶⁻²⁹ Several investigators have explored the effect of off-atom charge sites on the accuracy of charge-fitting procedures,^{19,29} and have concluded that adding some atomic anisotropy can noticeably improve the charge description. Lone pair containing models of

the water molecule for use in liquid simulations have also been proposed, notably ST2,³⁰ which contains two tetrahedrally oriented lone pairs, and TIP4P,¹⁷ which includes a single off-atom charge site on the hydrogen side of the molecule. We earlier showed that in a model of water with lone pairs, if the position of the lone pairs is optimized, the lone pairs invert to a TIP4P-like orientation,³¹ which was also found by Popkie et al.³² The water models with lone pairs have proven useful, but are not dramatically more effective than water models without lone pairs.¹⁷ As noted previously, an early version of the AMBER force field¹² included lone pairs on sulfur atoms, which have not been retained in the current version.⁷ The MM2³ force field included lone pairs on *sp*³-hybridized oxygen and nitrogen, which were abandoned in the MM3⁴ force field in an effort to accurately reproduce vibrational frequencies. In some of the above cases, the inclusion of lone pairs was found to be useful, but was eventually superseded by other improvements in force field design, such as larger training sets, availability of higher level *ab initio* charges for more substantial molecules and molecular fragments, and longer simulation times for model testing and validation. In many ways, some of the earlier force fields had not yet reached the limit of accuracy inherent in the chosen functional form. Thus, investigators were not in a position to assess the importance of inclusion of off-center charges, because the atom-centered model had not been made as accurate as possible. Recent trends in force field development efforts⁷ seem to suggest that this limit is being approached. It is in this spirit that the current investigation is undertaken. We have only analyzed the effect of lone pairs on the Cornell et al.⁷ force field, because for force fields involving empirically derived charges,³⁻⁶ it would not be possible to separate the effect of "adjusting" atom-centered charges from adding lone pairs. In this context, we will also evaluate

the effect of explicitly including polarization effects as well as considering nonatomic-centered charges.

As noted, the inclusion of lone pairs should provide some immediate benefit independent of force field performance. Because the charges used are derived by means of a statistical fit to the quantum mechanical molecular electrostatic potential, the addition of degrees of freedom should result in a better fit. As far as force field performance is concerned, it is hoped that treating specific atom types as multicentered, multicharged objects will result in significant improvement in model performance. The assessment of this improvement will proceed first by surveying a group of small molecule, hydrogen bonded systems. From these studies a preliminary set of parameters will be derived and will be tested in more realistic situations. In particular, the relative solvation free energies of the pyridine/benzene and the methanol/methane systems will be obtained.

Theory and Methods

The first issue that must be resolved is the number and location of additional charge sites for the various molecules in the test suite. Using the pyridine...HOH system of Figure 1 as the primary test case, many different atomic/extra site configurations were examined. Both one and two extra site models were considered, and it was found that a single extra site, in the plane of, and facing "out" from, the C—N—C angle was most effective in reproducing the quantum mechanical energy change. Having determined the number of extra sites and their approximate location, only the N—LP distance needs to be determined. At first, a value of 0.5 Å was obtained using two independent methods, although all values in the range of 0.3 and 0.7 Å gave good fits. The first was an empirical fit to the differential quantum mechanical energy in Figure 1, and the second was an optimization of the extra site position with respect to the statistical accuracy of reproduction of the electrostatic potential. The 0.5-Å extra site bond distance was initially chosen for all second row elements regardless of hybridization. In the case of sulfur, a better fit to the potential was found with this distance increased to 0.75 Å. Several of the molecules in the test suite were studied with these standard values, with promising success. Before proceeding with a more thorough survey, an analysis of the charge density³³ of many of the

molecules in the test suite was performed to more accurately ascertain the "best" general values for the X—LP distances to be used. These studies suggested that X—LP distance should be slightly shorter than the values noted previously. The O—LP and N—LP distances were reset to 0.35 Å and the S—LP distance to 0.7 Å. All of the distances for a particular element were very similar, regardless of hybridization, suggesting that the lone pair distance could be a transferable parameter. For this study, this has been taken as an assumption, with all lone pairs being treated in the same fashion.

Quantum mechanical calculations were carried out using the GAUSSIAN92³⁴ system of programs, and the density functional program DeFT.³⁵ Geometry optimizations were performed at the 6-31G*¹⁶ level of theory. Single-point energies were obtained at the MP2³⁶ level of theory for the 6-31G*-optimized structures. Counterpoise corrections were not applied to improve the quality of the absolute interaction energies because, at this stage, the main concern is with energy differences. Electrostatic potential data were generated at the 6-31G* level for use with the standard, additive force field model, and with a triple- ζ plus polarization DFT treatment,³⁷ for use with a polarizable force field model.

Point charges for all sites were determined by means of the RESP¹⁴ methodology using electrostatic potential data generated at the levels of theory noted previously. The quality of the derived charges was measured by means of the standard error:

$$\Sigma = \sqrt{\frac{\chi^2}{N_{esp}}} \quad (2)$$

where χ^2 is the merit function from the least-squares fit, and N_{esp} is the number of grid points at which the electrostatic potential was derived.

To incorporate the additional charge sites into the molecular dynamics calculations, we treated them as formal particles. To ensure that the lone pairs would remain relatively fixed, the force constants for bond stretching and angle bending were approximately doubled from those of standard hydrogen atoms types. However, to ensure that motions involving hydrogen atoms remain the highest frequency motions present, the lone pair was given a mass of three. This should ensure that conventional wisdom with regard to protocol selection (i.e., the use of SHAKE³⁸), choice of step size, length of simulation, etc., should apply un-

changed to molecular dynamics simulations involving lone pair systems. Although lone pairs can be incorporated into models by forcing them to have a fixed position relative to the heteroatom,³⁹ for testing the model we adopted the simpler approach of assigning mass and force constants to the lone pair. The complete list of parameters for the lone pair atom type are shown in Table I. This may effect dynamics but not equilibrium properties. Several approaches deliberately vary the atomic masses in the molecular system to improve sampling behavior.^{40,41} Thus, our use of this approach in solvation free energy studies of pyridine and methanol is justifiable.

Solvation free energies and molecular mechanics energies were obtained by means of the AMBER molecular dynamics program,⁴² employing the Cornell et al. force field.⁷ The free energy simulations were carried out over a 200-ps time scale with a 2-fs time step. SHAKE was applied to all bonds, electrostatic decoupling was employed, and the pmf correction was included to account for bond shrinkage. Initial solvated structures were equilibrated for 40 ps prior to the free energy simulations. To assess the error inherent in these calculations, the simulations were run in reverse, preceded by 20 ps of equilibration at the final structure from the forward simulation. Errors reported are the difference between the forward and backward simulations.

Results and Discussion

A set of 21 molecules was chosen as an initial training set to test the effect of lone pairs on force

field performance. There are examples of molecules containing *sp* nitrogen and *sp*², *sp*³, and aromatic nitrogen, oxygen, and sulfur atoms. The first issue regarding the effect of lone pairs on these systems is the quality of the charge derivation. In Table II, data are presented that address this question. For each molecule considered, electrostatic potential field data have been generated using two quantum mechanical methods. The first is a Hartree-Fock scheme and the second is a density functional method. The Hartree-Fock calculation involves the now standard 6-31G* basis set, whereas the density functional calculation employs triple- ζ plus polarization basis set on heavy atoms and a double- ζ plus polarization on hydrogen. This basis set has been shown to well reproduce experimental gas-phase dipole moments.³⁷ For both of these potentials, RESP point charges are derived for each molecule both with and without lone pairs. In each of these cases, the lone pairs were placed in the chemically intuitive positions. The molecules considered, along with a schematic representation of the explicit lone pairs included for each case, and the charge values obtained are available as Supplementary Material. The resulting dipole moments and standard errors from these calculations are reported, as well as the quantum mechanical and experimental dipole moments. As can be seen from these data, in almost every case, inclusion of lone pair sites improves the quality of the charge fit. The amines and dimethylether show a consistent worsening of the charge fit, whereas ammonia and acetamide show a slight decrease in accuracy for the 6-31G* potential. In all of these cases, however, this loss of accuracy was due to the extra constraints associated with the RESP charge-fitting process, either the harmonic restraint or the process of equivalencing interchangeable atoms. Upon removal of all constraints, every molecule showed improvement in the charge-fitting accuracy due to the inclusion of lone pairs. This is consistent with purely statistical considerations. It has been noted⁴³ that chemical lone pairs are often not the most effective means of improving the quality of derived charges. Although this may be true from a purely statistical point of view, the most important criterion to evaluate is force field performance. It seems more reasonable, therefore, to stay with a physically justifiable picture of molecular structure. However, many nonstandard charge distributions were evaluated with the result that the chemically intuitive lone pair structures always performed better in force field calculations. This is undoubtedly due to the fact that optimal hydrogen

TABLE I. Parameters Used for Lone Pair (LP) Atom Type.

Parameter	Value
Mass	3.0 amu
$r_{eq}(X-LP)$, $X \in N, O$	0.35 Å
$r_{eq}(X-LP)$, $X \in S$	0.70 Å
$K_r(X-LP)$, $X \in N, O, S$	600.0
$\theta_{eq}(LP-X-Y)$, $X \in sp^2 N, O, S$	120.0°
$\theta_{eq}(LP-X-Y)$, pyridine	120.0°
$\theta_{eq}(LP-X-Y)$, imidazole, furan, oxazole	126.0°
$\theta_{eq}(LP-X-Y)$, $X \in sp^3 N, O$	109.5°
$\theta_{eq}(LP-X-Y)$, $X \in sp^3 S$	90.0°
$K_\theta(LP-X-Y)$, $X \in N, O, S$	150.0
r^*	0.00
ϵ	0.00

TABLE II.
Charge Fitting Data^a.

Molecule	Par	6-31G*	HF	HF, LP	DFT(tz)	DFT	DFT, LP	Expt. ^b
Pyridine	μ	2.3201	2.2981	2.3163	2.2041	2.1702	2.1838	2.19
	std. err $\times 10^{-3}$	—	1.82	0.85	—	2.24	1.51	—
Imidazole	μ	3.8625	3.8096	3.8595	3.6728	3.5976	3.6421	3.87
	std. err $\times 10^{-3}$	—	2.86	1.84	—	2.98	1.98	—
Oxazole	μ	1.5833	1.5546	1.5814	1.6194	1.6016	1.6107	—
	std. err $\times 10^{-3}$	—	3.40	1.87	—	3.37	1.88	—
Methanimine	μ	2.2435	2.2210	2.2180	1.9760	1.9232	1.9422	1.971
	std. err $\times 10^{-3}$	—	1.89	1.75	—	2.24	1.74	—
Methyliminomethane	μ	1.7372	1.8135	1.9469	1.4847	1.6413	1.7746	1.53
	std. err $\times 10^{-3}$	—	2.83	2.15	—	3.40	2.80	—
Hydrogen cyanide	μ	—	3.1693	3.2015	—	2.8962	2.9315	2.95
	std. err $\times 10^{-3}$	—	1.12	0.42	—	1.41	0.73	—
Ammonia	μ	1.9189	1.9552	1.9610	1.5304	1.6074	1.5958	1.47
	std. err $\times 10^{-3}$	—	2.66	2.74	—	4.07	3.91	—
Methylamine	μ	1.5337	1.7659	1.9222	1.3120	1.6165	1.7357	1.30
	std. err $\times 10^{-3}$	—	3.85	3.97	—	4.32	4.63	—
Dimethylamine	μ	1.1421	1.4540	1.7053	0.9506	1.3600	1.6692	1.03
	std. err $\times 10^{-3}$	—	3.58	4.03	—	4.16	5.06	—
Trimethylamine	μ	0.7440	0.9859	1.2375	0.4179	0.7646	1.2088	0.63
	std. err $\times 10^{-3}$	—	2.56	3.49	—	3.15	5.31	—
Methanol	μ	1.8674	1.9664	2.0038	1.6056	1.6917	1.7315	1.662
	std. err $\times 10^{-3}$	—	2.82	2.62	—	3.31	3.08	—
Dimethylether	μ	1.4777	1.6240	1.7030	1.1434	1.3089	1.4247	1.30
	std. err $\times 10^{-3}$	—	2.12	2.31	—	2.58	2.95	—
Formaldehyde	μ	2.6642	2.6622	2.6823	2.2003	2.1941	2.2110	2.33
	std. err $\times 10^{-3}$	—	1.30	0.82	—	1.77	1.05	—
Furan	m	0.7717	0.7447	0.7831	— ^c	—	—	0.66
	std. err $\times 10^{-3}$	—	2.72	1.91	—	—	—	—
Formamide	μ	4.1010	4.0844	4.1145	3.8142	3.7856	3.8129	3.73
	std. err $\times 10^{-3}$	—	1.87	1.39	—	1.79	1.38	—
Acetamide	μ	4.0586	4.0543	4.1019	3.7640	3.8033	3.7943	3.76
	std. err $\times 10^{-3}$	—	1.21	1.33	—	1.28	1.25	—
<i>N</i> -methylacetamide	μ	4.0424	4.1065	4.1495	3.7563	3.8193	3.8486	3.73
	std. err $\times 10^{-3}$	—	1.72	1.64	—	1.97	1.82	—
Methanethiol	μ	1.7875	2.0319	1.9422	— ^d	—	—	1.52
	std. err $\times 10^{-3}$	—	4.56	3.51	—	—	—	—
Dimethylthioether	μ	1.7969	1.5510	1.7398	— ^d	—	—	1.50
	std. err $\times 10^{-3}$	—	6.05	4.98	—	—	—	—
Methanethial	μ	2.2325	2.2788	2.3074	— ^d	—	—	1.647
	std. err $\times 10^{-3}$	—	3.76	3.60	—	—	—	—
Thiophene	μ	0.8973	0.8869	0.9122	— ^d	—	—	0.55
	std. err $\times 10^{-3}$	—	2.30	1.86	—	—	—	—

^a All dipole moments in debyes and standard errors in e^-/bohr^3 .^b From Ref. 55.^c DeFT calculation failed to converge.^d DeFT basis set not available for sulfur.

bonding configurations almost always correspond to maximized donor/lone pair interactions.⁴⁴ As will be presented in what follows, inclusion of lone pairs will not always dramatically improve force field performance, but in no case was an atom-

centered or counterintuitive lone pair model superior to the chemically intuitive lone pair model.

As noted previously, there have been other studies that have attempted to include off-atom charge sites in statistical fits of molecular electro-

static potentials. Some of these address issues pertinent to the current line of investigation and deserve some comment, particularly in view of the fact that our approach yields somewhat different results. The investigation of optimal lone pair sites in a series of azabenzene molecules, undertaken by Williams and Weller,^{23,24} indicated that lone pairs were essential to describe accurately the crystal structures of these compounds with molecular packing analysis (MPA). However, the optimal N–LP distances were considerably shorter than the 0.35-Å proposed here. In that study, most molecules had optimal off-atom charge sites within 0.1 Å of the nitrogen atom. We have performed the same analysis of the six azabenzene molecules presented in that study with respect to our RESP charge-fitting procedure. In our case, we find that optimal N–LP distances, with respect to the rms fit to the electrostatic potential, are in the range of 0.40 to 0.50 Å. We can only conclude that choice of basis set, external point selection criteria, and the particular features of the RESP algorithm account for the difference. It should be noted that, for the practical application of crystal packing calculations undertaken by Williams and Weller, the N–LP distance was uniformly lengthened to 0.25 Å, in spite of indications of the charge fit results. The modest increase in the observed rms fit error this choice imposed was justified by the quality of the crystal packing calculations and the more intuitively reasonable charge values obtained. We also wish to use the performance of the model in force field calculations as our main criterion for quality assessment. This is not to say that we are not concerned with the most effective way to reproduce the quantum mechanically calculated molecular electrostatic potential—quite the contrary. However, a highly accurate potential model which performs poorly in force field calculations is of little value for our current investigations.

The other major body of work investigating inclusion of off-atom charge sites in molecules centers around the water molecule. As noted previously, optimization of lone pair positions in the water molecule lead to a collapse of off-atom sites into a single site, usually located along the C_{2v} axis on the hydrogen side of the molecule. Greater success in developing improved solvent models for water that include off-atom sites has been noted for those models which place a single site within the H—O—H angle rather than two arranged tetrahedrally. We have explicitly investigated this lone pair arrangement in the sp^3 oxygen systems included in our study, methanol and dimethyl

ether, and found that the tetrahedral arrangement is at least as good as, if not superior to, the standard single-site model in force field calculations. We have attempted to optimize the lone pair position with respect to the fit the electrostatic potential, and did not observe the lone pair collapse which is usual in the water calculation. The optimal arrangement from this criterion was tetrahedral, with an O–LP distance of 0.45 to 0.50 Å, although a fairly broad range of distances resulted in only minor increases in the observed fit error. A single-site model for sp^3 oxygen would probably work for the molecules considered; however, there is no compelling reason from our initial investigations to suggest that this is the preferred model for any molecule other than water. Indeed, it may be that water is an unique model subject, and that the requirements of a good solvent model for molecular simulations lead to the improved performance for single off-site models. In our investigations, which do not include water or any neat solvent calculations, we have chosen to stay with the chemically intuitive lone pair orientation for all molecules considered, including those containing sp^3 -hybridized oxygen.

As noted previously, the most important question to be answered is how the inclusion of lone pairs affects force field performance. As a first simple step toward this end, all of the small molecules considered here were optimized in the gas phase, both with and without lone pairs. The results of these calculations are available as Supplementary Material. In addition to the standard molecular mechanics model, a methodology incorporating atomic polarizabilities⁴⁵ was also employed. The atomic polarizabilities employed in this model are taken from Applequist et al.,⁴⁶ and do not include a value for sulfur. A value of 1.25 Å³ was determined by noticing that changing oxygen to sulfur (e.g., H₂O to H₂S) increases the molecular polarizability by a factor in the 2.5 to 3.0 range.⁴⁷ Therefore, the oxygen atomic polarizability presented by Applequist et al., approximately 0.47, increased by the aforementioned factor, yields a sulfur polarizability between 1.18 Å³ and 1.41 Å³. The final value was chosen as a compromise between these two values.

The point charges used for the standard model were derived from the 6-31G* potential, whereas the polarizable model charges were obtained from the DFT-generated field. The exceptions to this rule include furan, for which the DFT calculation failed to converge, and the sulfur compounds, for which there is no basis set available. In these cases,

the 6-31G* potential charges were scaled by 0.88 for use in the polarizable model.^{48,49} The standard reasoning behind this distinction in charge models is that 6-31G* already contains some “implicit” polarization as demonstrated by its consistent overestimation of dipole moments (see Table II). This can be beneficial in aqueous solution calculations which do not explicitly include a polarization term, particularly in view of the fact that the TIP3P and SPC water models include such “implicit” polarization. In the case of the polarizable model, however, this overestimation of the dipole moment leads to error if the resulting charges are used unchanged. This is the reasoning behind the choice of an unusually large basis set for the DFT calculation performed; that is, the calculated dipole moments are much more consistent with gas phase experimental values.

For each of the molecules considered in this study, energies associated with both optimal and suboptimal hydrogen bonded systems were obtained. In every case, both the quantum mechanical and molecular mechanical models had nearly

linear X···H—O angles. The X···O distances were similar among all the models with the molecular mechanics treatments resulting in slightly shorter distances, on the order of 0.2 Å, than the quantum mechanical results. The particular conformations considered are presented in Table VI. In all cases, the hydrogen bond donor was a water molecule, specifically at TIP3P¹⁷ water molecule for the additive force field calculations, and a POL3⁴⁸ water molecule for the polarizable force field calculations. In addition, the formamide dimer was considered as a representative of the type of hydrogen bonding found in protein structures. The results of these calculations are summarized in Table III and reported in full in the Supplementary Material. The results can best be summarized by considering atom types and hybridizations of the acceptor atoms as families. Comparison with *ab initio* energies refers to the MP2/6-31G*//6-31G* values and, in the case where three conformations have been reported, the best and worst are compared. Even though comparisons are being made against

TABLE III. Relative Conformational Energy Data.^a

System	6-31G*	MP2 ^b	AMBER ^b	AMBER POL ^b	AMBER LP ^b	AMBER POL–LP ^b
Pyridine···HOH	−3.31	−3.94	−0.44	−0.64	−3.51	−5.44
Imidazole···HOH	−2.81	−3.03	+0.22	+0.58	−3.52	−4.72
Oxazole···HOH (N-acceptor)	−2.97	−3.18	−0.85	−0.44	−3.34	−5.13
Oxazole···HOH (O-acceptor)	−1.75	−1.77	−1.28	−0.79	−2.83	−3.22
Methanimine···HOH	−2.92	−3.53	−1.48	−1.55	−3.83	−3.75
Methyliminomethane···HOH	−3.26	−3.81	−0.53	−0.71	−3.27	−4.61
Hydrogen cyanide···HOH	−1.24	−1.53	+0.19	+0.04	−1.11	−1.50
Ammonia···HOH	−3.53	−4.06	−2.30	−2.16	−4.87	−3.67
Methylamine···HOH	−4.07	−4.90	−2.98	−3.31	−5.05	−4.35
Dimethylamine···HOH	−5.55	−7.13	−5.10	−4.07	−6.60	−6.54
Trimethylamine···HOH	−5.50	−7.49	−3.40	−2.88	−4.38	−4.13
Methanol···HOH	−1.29	−0.76	−1.40	−1.10	−1.52	−1.43
Dimethylether···HOH	−1.41	−0.79	−0.87	−0.64	−1.01	−0.91
Formaldehyde···HOH	−2.46	−3.12	−1.03	−0.99	−1.97	−2.16
FutAn···HOH	−0.95	−0.82	+0.63	+1.14	−1.43	−0.82
Formamide···formamide ^c	−3.42	−2.24	−2.04	−0.90	−2.78	−1.76
Formamide···HOH	−2.84	−3.54	−1.15	+0.06	−3.50	−2.03
Acetamide···HOH	−2.37	−2.69	−1.53	−0.51	−3.15	−1.86
N-methylacetamide···HOH	−1.96	−2.36	−0.86	−0.77	−2.94	−3.08
Methanethiol···HOH	+1.01	+1.31	+0.44	+0.34	+1.29	+1.58
Dimethylthioether···HOH	+1.05	+1.41	+0.28	+0.33	+1.59	+1.54
Methanethial···HOH	−1.86	−2.32	−0.86	−0.69	−0.98	−0.78
Thiophene···HOH	+0.49	+0.48	+0.92	+1.07	+0.90	+0.99

^a All energies in kilocalories per mole representing difference between best and worst conformers in Table VI.

^b Hartree–Fock energies obtained at the level of theory noted with 6-31G* structures. AMBER calculations performed using the force field in Ref. 7.

^c Structure constrained to single H-bond conformation.

TABLE IV.
Overall Errors in Model Performance^a

MM model	E (interaction, optimal) ^b		ΔE (conf.) ^c	
	MP2	6-31G*	MP2	6-31G*
Average absolute error				
AMBER 4 / TIP3P	1.313	0.843	1.711	1.305
AMBER 4 POL / POL3	1.958	0.940	1.985	1.602
AMBER 4-LP / TIP3P	0.469	1.541	0.654	0.617
AMBER 4 POL-LP / POL3	1.068	1.057	1.014	1.017
Root mean square error				
AMBER 4 / TIP3P	1.579	1.632	2.021	1.538
AMBER 4 POL / POL3	2.232	1.361	2.286	1.826
AMBER 4-LP / TIP3P	0.758	1.745	0.921	0.700
AMBER 4 POL-LP / POL3	1.428	1.511	1.383	1.264

^a All energies in kilocalories per mole.
^b Absolute interaction energy of optimal configuration.
^c Relative conformational energy between best and worst conformers (see text).

absolute interaction energies, it is clear that these values are not the most reliable. Absolute interaction energies can be difficult to calculate accurately.⁵⁰ For this reason, errors are reported in Table IV against both MP2 and 6-31G* values. From these data, one can get an overall sense of the improvement in model performance due to the inclusion of lone pairs. Because of the fact that the *ab initio* calculations at the 6-31G* and 6-31G*/MP2 levels of theory exaggerate the hydrogen bond energies, particularly when the counterpoise correction is not applied, our goal is not to reproduce these values. We do consider it significant, however, that the models with lone pairs do significantly improve the relative conformational energies which do more accurately reflect hydrogen bond directionality. Overall improvement is about a factor of two for predicting hydrogen bond directionality. These results will be examined in more detail.

NITROGEN ACCEPTORS

For aromatic nitrogen, specifically pyridine imidazole, and oxazole, the inclusion of a single lone pair dramatically improved discrimination between the hydrogen bonded conformations studied. In these cases, the optimal hydrogen bond was along the N-LP vector whereas the suboptimal configuration was perpendicular to the plane of the molecule. The *sp*² nitrogen-containing system, methanimine and methyliminomethane, also exhibited a dramatic improvement through the addition of lone pairs. The structures considered in these instances were the same as the aromatic example. The optimal hydrogen bond was along the N-LP axis and the suboptimal structure was perpendicular to the plane of the molecule. For the one *sp* nitrogen compound considered, hydrogen cyanide, the addition of a single lone pair also had a dramatic effect. The standard models incorrectly

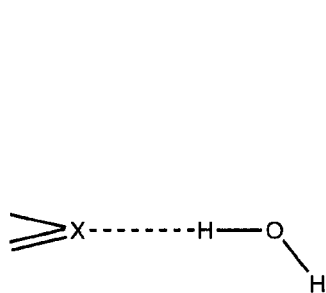
TABLE V.
Free Energy Results for Pyridine and Methanol Simulations.^a

System	ΔΔG (calc.)	ΔΔG (expt.) ^b	Diff.
Pyridine(lp) → benzene	3.95 ± 0.05	3.90	0.05
Pyridine → benzene	2.28 ± 0.02	3.90	1.62
Methanol(lp) → methane	7.14 ± 0.01	7.11	0.03
Methanol → methane	6.86 ± 0.01 ^c	7.11	0.25

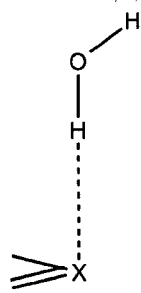
^a All energies in kilocalories per mole.
^b From Ref. 56.
^c From Ref. 57.

TABLE VI.
Hydrogen Bond Conformations.

sp N, *sp*² N and aromatic N,O,S

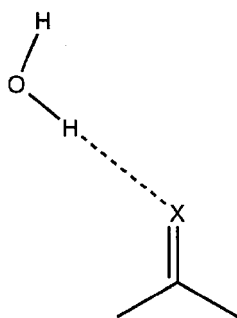


form I



form II

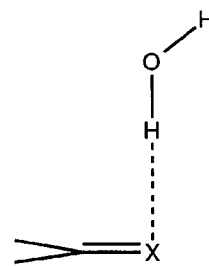
*sp*² O,S



form I

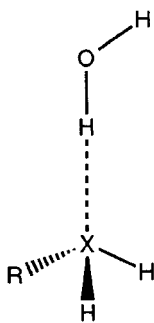


form II

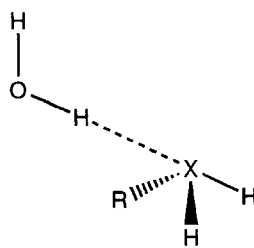


form III

*sp*³ N

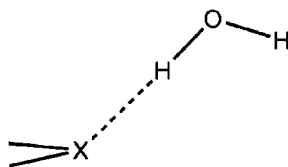


form I

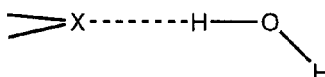


form II

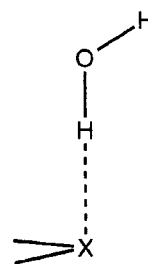
*sp*³ O,S



form I



form II



form III

predict the minimum energy structure, whereas those with the lone pair correctly mimic the *ab initio* data.

The case of sp^3 nitrogen presents a set of mixed results. Methylamine and dimethylamine show dramatic improvement in both the absolute interaction energy of the optimal conformation as well as the conformation energy difference. Performance of the model for ammonia and trimethylamine was not as positive. In the case of ammonia, the charge on the lone pair was positive, and led to a smaller hydrogen bond directionality than found quantum mechanically. This may be analogous to TIP4P water in which the extra charge is located "inside" the HOH angle; that is, simply moving the charge off of the heteroatom is more effective than adding more total charge sites. Noticeably improved results in this case are obtained by forcing the charge on the nitrogen atom to be zero for the lone pair models. This yields much better interaction and relative conformational energies, and represent the results presented in Table III and the Supplementary Material. The case of trimethylamine does not permit such a simple solution. Overall interaction energies and conformational energies are too low for both the additive and polarizable models. The source of the difficulty seems to be in the charge fitting procedure. The standard model employed imposes extra constraints on methyl and methylene groups. In a molecule such as trimethylamine, there is very little flexibility left in the fitting procedure. In this case, the limitation results in a charge on the lone pair which is small compared with that found in methylamine and dimethylamine, -0.45 rather than -0.6 electrons. Lengthening of the N-LP bond results in improved model performance, but reproduction of the *ab initio* data requires impractical bond distances. The model performance can also be improved by fixing the nitrogen and lone pair atomic charges at the values obtained for the other amines. The interaction energies and relative conformational energies still fall short of the *ab initio* values, however, and it was decided that the model would not be fine-tuned further to treat this molecule more accurately. It should be pointed out, however, that the inclusion of lone pairs does improve model performance; it is quantitative agreement with quantum mechanical data which is currently at issue. Some of these difficulties in developing amine charge models may be related to the difficulties in reproducing the free energy of solvation of amines.^{51,52}

In comparing the performance of the additive and the polarizable force field models overall, one can see that, generally, smaller interaction energies, and often the relative conformational energies as well, are obtained with the polarizable model. However, for the aromatic nitrogen acceptors, the relative conformational energies are noticeably larger with the polarizable model. As in the case of trimethylamine, this anomaly can be traced to the charge-fitting procedure. The magnitude of the charges in these molecules is rather large compared with both the 6-31G* RESP charges and the DFT RESP charges in the absence of lone pairs. These large charges destabilize the suboptimal conformations too significantly. The cause of this seems to be that the lone pair is too close to the nitrogen atom for these molecules and this basis set. The N-LP distance was determined by analyzing the 6-31G** charge density, not the triple- ζ DFT charge density. In addition, recall that preliminary studies of pyridine suggested an N-LP distance longer than the one used here. Lengthening of this bond does indeed result in improved model performance in these cases. Improved performance can also be obtained by fixing one charge value; for instance, the charge on the nitrogen, to some reasonable value. The resulting charges will then be more consistent with the expectation of the force field, specifically, that charges used for the polarizable model should be "smaller" than those used for the additive model (cf. the previous discussion of basis set and charge derivation). As in the case of trimethylamine, results have been reported for the standard model, rather than those associated with any individual adaptation. The standard model does an excellent job on the absolute interaction energies of the optimal conformations, and should prove useful in further calculations. Knowledge of potentially significant refinements, however, may prove useful if a more specific force field treatment is required.

OXYGEN ACCEPTORS

Like nitrogen hydrogen bond acceptors, oxygen acceptors play important roles in biochemical structure and function. Two examples of aromatic oxygen have been included in the test suite, furan and oxazole. In both cases, the lowest energy configuration places the hydrogen bond along the O-LP axis. The addition of a single, lone pair to the oxygen atom results in noticeable model per-

formance improvement. In particular, the standard force field approach incorrectly predicts the lowest energy conformation in the case of furan. The addition of a lone pair reverses this prediction and much more closely mimics *ab initio* results. As mentioned previously with respect to the oxazole nitrogen acting as an acceptor, the charges on oxazole can be unusually large, particularly those derived from the DFT electrostatic potential. As in the case of nitrogen, fixing the acceptor atomic charge to some reasonable value leads to much improved results. As has been the practice up to this point, the results of the standard lone pair model are reported in Table III Supplementary Material, and individual solutions to particular model shortcomings are treated separately.

The one example of pure sp^2 oxygen considered, formaldehyde, reflects marked improvement with the inclusion of lone pairs. In this case, the relative conformational energy is approximately doubled with respect to the results without lone pairs. This energy is still lower than expected, but most of the error has been removed. Further improvement can be achieved with a lengthening of the O–LP bond.

As was the case with nitrogen, sp^3 -hybridized oxygen acceptors show mixed results. As was also the case previously, these model shortcomings can be traced to the quality of the charge fit. For methanol, the addition of lone pairs improves the interaction energy associated with the lowest energy conformer. The relative conformational energies are already larger than the *ab initio* values without the lone pairs. Adding these into the system increases the relative energies further. The error is still less than 1 kcal; however, it is clear that addition of the lone pairs has pushed this energy in the wrong direction. The case of dimethylether exhibits almost no effect due to the addition of lone pairs to the system. The optimal configuration interaction energy is still low and almost identical to the value without lone pairs. This is a situation similar to trimethylamine discussed previously; that is, there is little flexibility in the charge fitting procedure. In this case, the magnitude of the resulting charges is particularly small. Simply scaling the charges up results in better model performance overall, at the expense of the calculated dipole moment. As a special case, this seems like a reasonable solution; however, it would be more satisfying for charges among atom types to be more consistent. The results presented in Table III and Supplementary Material were generated using unscaled charges. Lone pairs can be

useful for oxygen sp^3 systems, but, at this point, they must be applied with care. It should be noted that hydrogen bonding interactions with carbonyl and ether oxygen atoms have “soft” bending potentials.⁵³ This can make selection of optimal configurations challenging from the standpoint of molecular mechanics, particularly in light of the restriction that lone pair orientations are fixed.

The amide functionality is of crucial importance in biochemical systems, so it is of particular interest to assess the model’s performance in these cases. In all of the amide \cdots HOH systems considered, significant benefit accrued from the inclusion of lone pairs. Both the interaction energies and relative conformational energies are much more consistent with quantum mechanical calculations. In addition, the performance of the model on the formamide \cdots formamide system improved noticeably with lone pairs. In this case, the global minimum structure is a cyclic structure involving two hydrogen bonds. This is also the case in the formamide and acetamide \cdots HOH systems. However, we wished to limit consideration, at this stage, to singly hydrogen bonded species. Therefore, the optimal structure for the formamide dimer was restrained to the single hydrogen bond structure illustrated in Figure 2. The suboptimal structure used here fixes the $O\cdots H-N$ angle at 90° . The success of this model on the amide systems suggests that lone pairs could contribute significantly to the accuracy of protein modeling studies.

SULFUR ACCEPTORS

The cases involving sulfur atoms seem to follow the trend exemplified by the oxygen systems. The

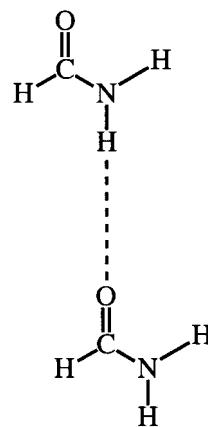


FIGURE 2. Single hydrogen bonded configuration of the formamide dimer.

aromatic sulfur acceptor considered, thiophene, shows improvement when lone pairs are added to the system. The energy improvements are quite small, however, they are in the right direction. In the sp^2 -hybridized case, methanethial, modest improvement in both interaction and relative conformational energies is seen. The relative conformational energy is still low relative to the quantum mechanical standard and can be improved by increasing the magnitude of the atomic charges. Like the trimethylamine and dimethylether cases discussed previously, methanethial charges seem rather small. Correcting this leads to further improvement in model performance.

The sp^3 -hybridized systems, methanethiol and dimethylthioether, show improvement in the interaction energy of the optimal conformer. However, the relative conformational energies do not improve, and the overall trend among the three conformers is not reproduced. This is particularly pronounced in dimethylthioether where the difference between conformers I and III should be very close to 0.0 kcal/mol; that is, the 90° conformer should be of lowest energy, with the tetrahedral arrangement very close energetically. However, the energy difference turns out to be significant. A solution to this is to move the lone pairs from the tetrahedral position to perpendicular to the C–S–C plane. Previous studies have found this arrangement of lone pairs to be superior to a tetrahedral arrangement as well.^{12,44} In this manner, the overall trends exhibited in the quantum mechanical calculations are preserved in the force field calculations, and the interaction and conformational energies are reproduced. In view of the clear superiority of this arrangement of lone pairs for sp^3 -hybridized sulfur, these results are reported in Table III and Supplementary Material.

FREE ENERGY CALCULATIONS

Calculations on simple hydrogen bonded complexes are very important in the development and assessment of force field performance. However, it is essential that the method be applicable to condensed phase systems as well. In particular, it is necessary to insure that the changes due to the introduction of lone pairs has not changed the effectiveness of the original force field. One of the most important ways to assess force field simulations in condensed phases is to evaluate the ability to calculate relative free energies. This type of calculation should provide an important test of the new molecular description. The relative solvation

free energies of pyridine with respect to benzene and methanol with respect to methane have been calculated. The results of these simulations are reported in Table V. The addition of the lone pair on the nitrogen of pyridine results in a dramatic improvement in the calculated solvation free energy. This is particularly satisfying in light of the improvement shown in the specific pyridine...HOH interaction. The methanol simulation results are also encouraging. In this case, additional lone pairs provided only modest improvement in the specific methanol...HOH interaction energies, and the standard force field does a very good job describing the solvation energy. However, the simulation with the lone pairs present showed improvement over those without. In spite of the excellent agreement with experiment shown by the calculations involving lone pairs, the most encouraging aspect of these calculations is the relative changes due to lone pair inclusion. In the pyridine case, a large improvement was needed and was obtained. The methanol case required only a small improvement, which was observed. These results seem to indicate that parameters derived from the small molecule gas phase calculation can be effectively transferred to condensed-phase calculations. The quantitative agreement with experiment should simply be considered fortuitous. Indeed, it should be pointed out that much of the error inherent in the pyridine calculation without the lone pair can be eliminated by using atom centered ESP-fit charges, rather than RESP charges, derived from a much finer field grid. This approach yields much more accurate potential derived charges, with respect to reproducing quantum mechanical electric moments, which can improve the calculated solvation free energy.⁵⁴ These charges do not perform as well as the RESP charges with respect to interaction energies, however. This point is made to indicate that there are other methods by which particular shortcomings of the force field may be addressed. It is our hope that lone pairs provide a more general improvement to the force field, applicable to a wide variety of problems. The free energy results presented here are a positive endorsement for our choice to include lone pairs.

Conclusion

In this study we have assessed the role of inclusion of lone pairs as well as explicit polarization on force field performance. The effect of the addition

of lone pairs on force field performance has been seen to be positive overall. The addition of charge sites to a molecular framework leads to improved statistical accuracy of the potential derived charges. The choice of chemically intuitive lone pair positions for these additional charge sites is a very good first step and has resulted in dramatic improvement in discrimination between hydrogen bonded conformations. In one case, sp^3 -hybridized sulfur, the lone pair position was refined to be perpendicular to the X-S-X plane. In the few cases, where consistent agreement with reference quantum mechanical data was not observed, the differences are subject to eradication through a change in the parameters associated with the lone pair particle. In particular, careful attention to the values of the atomic charges often resulted in noticeable improvement over the "standard" model performance. In no case did the addition of lone pairs result in an incorrect minimum energy structure and, in three cases, hydrogen cyanide, imidazole, and furan, the standard force field was corrected. The success of the free energy simulations of pyridine and methanol suggests that the power and applicability of the force field have been retained, with the lone pairs providing increased accuracy.

The positive results presented here suggest that lone pairs, or some other method which incorporates anisotropy into the charge distribution of donor centers, deserve to play a role in future force field developments. The dramatic improvement observed should prove critical in any molecular mechanics investigation focusing on specific atomic interactions. In addition, the improved flexibility imparted to the polarizable force field model provides an excellent avenue for consistent model performance improvement. Because the lone pairs need to be added only at electron donor centers, the additional computational cost should be quite modest for simulation of macromolecules.

Further work in this area will concentrate on refining the methodology by which lone pairs are incorporated into the calculations. In addition, other problems of biochemical interest will also be addressed.

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